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THE RESPONSE OF SWINE TO AN OVINE PNEUMONIA VIRUS[1]

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McKercher *et al.* (1, 2) and Boidin, Cordy and Adler (3) reported the isolation of a virus of the psittacosis-lymphogranuloma group from pneumonic sheep lungs and the experimental reproduction of pneumonia in lambs. Because of the histopathologic similarity between the pneumonia caused by this virus in sheep and virus pneumonia of pigs (VPP), a study of the pathogenicity of the sheep virus for swine was undertaken.

This report deals with the results of infection experiments in which sheep pneumonia virus (Boidin's SPB strain (3)) was used as the infecting agent and pigs as the experimental animals. The experimental findings on symptomatology, necropsy, histopathology and microbiology of this study are hereby presented.

MATERIALS AND METHODS

Boidin's SPB strain of ovine pneumonia virus was used in all experiments. The virus was maintained by yolk sac passage. 5–7 day-old embryonating chicken eggs were used in the experiments.

Inoculation

Inoculums for pigs were prepared from yolk sacs harvested from dead embryos which had been inoculated with 0.25 ml of 1% infected yolk sac in tryptose broth. A 10% infected yolk sac suspension was then prepared, kept at 4 C overnight, and then the supernatant was used as the inoculum. Inoculums were checked for virus content by injecting 0.2 ml of 10-fold serially diluted materials into the yolk sac of embryonating eggs, six eggs being used for each dilution. The inoculum used for the first inoculation in Experiment II was prepared from infected yolk sacs which had been kept in the refrigerator (4 C) for 10 days, while all other inoculums were freshly prepared.

Inoculated eggs were examined twice daily. Rabbit blood agar plates and thioglycolate broth were used for detecting bacterial contamination.

Healthy Duroc, Chester-white and Hampshire breeds of pigs, weighing about 40 lb and without previous history of VPP or sheep pneumonia virus infection, were purchased from the clean herd of the University farm. During the 7-days observation period prior to inoculation, body temperature and chest stethoscopy were frequently done to ascertain that the pigs were free from pneumonia. Pigs were infected intratracheally with 5 ml of infectious material. Tryptose broth (pH 7.2) was used throughout as the diluent.

Fixation and Staining of Specimens

One or more pieces of swine lung tissue were taken from grossly healthy as well as obviously diseased areas of each lobe of the lungs. Lung tissue and tissues from other organs were fixed in 10% aqueous formalin solution. Paraffin sections were cut at 4 to 6 μ.

For routine examination, hematoxylin and eosin stain was used. Bielschowsky-Foot method was employed for staining the reticulum. The methods of Feulgen and Feulgen naphthoic acid hydrazine (Feulgen NAH), Pollack trichrome, periodic acid Schiff (PAS) and Macchiavello stains were used for histologic sections or smear preparations as necessity arose.

EXPERIMENTAL

Two infection experiments were performed as follows:

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Experiment I

6 pigs were used for this experiment. They were restrained on their backs with their heads held about 30° from the horizontal for the intratracheal inoculation. Inoculated pigs, selected at random, were killed for necropsy; two each at 4, 8 and 15 days after inoculation. Virus titer of the inoculum was $10^{6.0}$ ELD_{50}.

For control purpose, a normal pig was inoculated with 5 ml of 10% uninfected yolk sac suspension intratracheally, then killed and necropsied on the 2nd day after inoculation. Another pig, never inoculated, was also killed and necropsied as a normol control.

Experiment II

10 pigs were inoculated in the natural standing position. 2 pigs, both afebrile, were selected at random and killed for necropsy at 12 hours after inoclation. 8 pigs which exhibited fever were selected at random and killed for necropsy, two each at 1, 2, 3 and 21 days after inoculation. The virus titer of the inoculum was $10^{6.0}$ ELD_{50}.

2 pigs which were experimentally infected were given 2 more infectious doses at 14 and 74 days after the 1st inoculation. They were killed at 4 and 9 days after the 3rd inoculation. The virus titer of inoculums was $10^{6.6}$ ELD_{50}.

2 pigs were killed and necropsied as normal control for histopathological examination in this experiment.

RESULTS

In experimental pigs, there was an almost uniform progression of signs and lesions. The results obtained are separately described under symptomatology, necropsy, histopathology and microbiology. The results are summarized in Table I.

Symptomatology

The infected animals showed high temperatures ranging from 104.7 to 107.4 F on the 1st or 2nd day after inoculation. The arithmatic mean of the peak temperature was 106.3 F and the fever persisted for 1–3 days. Rapid and shallow breathing was noted within 24 hours after inoculation. Intermittent non-productive coughing or tracheal rales were observed in about half of the animals. The pigs refused to eat. These clinical signs disappeared rapidly follwing recovery from fever, except for coughing which usually lasted about 8 days after inoculation. Thereafter, animals appeared as normal as they did prior to inoculation, and quickly regained their appetite. No diphasic fever response was noted within the observation period of this study.

2 pigs which were inoculated again 14 days after the primary infections showed a febrile response 1 day after inoculation. The average temperature was 105.4 F. They recovered from fever within 3 days after inoculation. Only one animal showed transient non-productive coughing, which was slight as compared with the animals in Experiment I. Recovery to normal was also quick.

After the 3rd inoculation, these 2 pigs again showed a fever response within 24 hours following inoculation, ranging from 105.6 to 107.2 F. The mean peak temperature was 106.4 F, and the body temperature returned to normal on the 2nd day after inoculation. Coughing was seen in one pig on the 2nd day after inoculation, and lasted only one day.

No abnormalities were found in the pig inoculated with non-infected yolk sac suspension. Normal control pigs did not exhibit any abnormal signs prior to necropsy.

Pathology

The lung lesions developed uniformly in a pattern related to the time elapsed after inoculation.

Lesions at 12 Hours (*Case 938, 939*)

Focal, lobular, reddish, sunken, consolidated areas were observed here and there in the apical and intermediate lobes of both lungs. Small amounts of slightly cloudy, sticky mucus were found in the trachea and the bifurcation of the large bronchi. Slightly swollen pulmonary hilar lymph nodes were observed.

Microscopically, increasing numbers of moderately swollen and hyperchromatic nuclei of fibroblasts with sparsely infiltrated lymphocytes, eosinophils and neutrophils around blood vessels and bronchioles was the common change in the lungs. In the affected lobules, changes were found in only a small portion of each lobule, usually around the bronchioles. Infiltrating inflammatory cells, mostly neutrophils, and slightly hyperplastic septal cells were the common changes in the thickened alveolar walls (Fig. 1). Some partially collapsed alveoli contained neutrophils and desquamated alveolar lining cells. No significant congestion and edema were observed. Occasionally,

TABLE I

Virus inoculation and resulting lesions in experimental pigs

Expt. No.	Pig No.	Dose	Inoculation Route/ Position	Virus titer of Inoculum	Days Killed	Bacteriological Checking of lung	Lesions
Expt. I	776	5 ml	IT/Supine	$10^{6.0}$ ELD$_{50}$	4	Nagative	Bronchopneumonia, perivascular and peribronchiolar lymphoid hyperplasia
	777	〃	〃	〃	4	〃	
	799	〃	〃	〃	8	〃	Marked perivascular lymphoid hyperplasia and marked septal cell hyperplasia
	800	〃	〃	〃	8	〃	
	810	〃	〃	〃	15	〃	Marked peribronchiolar and perivascular lymphoid hyperplasia
	811	〃	〃	〃	15	〃	
Expt. II	938	〃	IT/Standing	〃	12	〃	Early stage of bronchopneumonia
	939	〃	〃	〃	12	〃	
	959	〃	〃	〃	1	〃	Acute bronchopneumonia
	960	〃	〃	〃	1	〃	
	961	〃	〃	〃	2	〃	Acute bronchopneumonia, moderate perivascular lymphoid hyperplasia
	962	〃	〃	〃	2	〃	
	963	〃	〃	〃	3	〃	Acute bronchopneumonia, perivascular lymphoid hyperplasia
	964	〃	〃	〃	3	A few colonies of hemolytic streptococci	
	1044	〃	〃	〃	21	Negative	Moderate peribronchiolar and perivascular lymphoid hyperplasia
	1095	〃	〃	〃	21	〃	
	1314*	〃 (1st)	SC	〃	4 days after 3rd inocul.	〃	Comparable to that of 8 days after inoculation
		〃 (2nd)	IT/Standing	〃			
		〃 (3rd)	〃	$10^{6.6}$ ELD$_{50}$			
	1349*	〃 (1st)	SC	$10^{6.0}$ ELD$_{50}$	9 days after 3rd inocul.	〃	Comparable to that of 21 days after inoculation
		〃 (2nd)	IT/Standing	〃			
		〃 (3rd)	〃	$10^{6.6}$ ELD$_{50}$			
Control	813	〃	IT/Supine	10% normal yolk suspension	2	〃	None
	813	None	None	—	—	—	None
	914	〃	〃				〃
	942	〃	〃				〃

Legend: IT: Intratracheal inoculation; SC: Subcutaneous inoculation.

*: Received 2nd and 3rd inoculums at 14 and 74 days after the 1st inoculation, respeectively.

tiny foci of necrosis, degeneration and desquamation of epithelial cells were observed in the small to medium-sized bronchioles. Hyperplastic epithelial cells were also noted in the portion adjacent to the damaged epithelial cells. Small amounts of neutrophils and alveolar epithelial exudates were found in the bronchial lumen. These changes never extended beyond the basement membrane of the mucosa. No significant changes were observed in the large bronchioles.

Moderate numbers of neutrophils were found in the lymph sinuses and in the lymph follicles of the pulmonary hilar lymph nodes. Moderate lymphoid hyperplasia and marked development of germinal centers also were observed.

Lesions at 1 Day (*Case 959, 960*)

Lobular, reddish, sunken foci were more frequently seen here and there than in cases at 12 hours. Most of the lesions were observed in the ventral part of the apical, intermediate and cardiac lobes. Linear, deeply sunken areas were found in the right apical lobe in case 959. A small amount of slightly cloudy, sticky mucus was found in the trachea and bifurcation of the large bronchi. The cut surfaces were particulary edematous in case 960. Few consolidated foci were found in the posterior lobes in either case. Slightly to moderately swollen and edematous pulmonary hilar lymph nodes were observed in both cases.

Microscopically, lobular involvement was charateristic. The bronchiolar mucosa was usually intact. Occasionally, tiny necrotic foci and desquamation of epithelial cells were found in the mucosa of the small bronchioles and the respiratory bronchioles. Moderate eosinophilic infiltration was seen in the lamina propria of the bronchioles in case 960. In the affected lobules, respiratory bronchioles and alveolar ducts were plugged with prominent cellular exudates, mostly neutrophils with some desquamated epithelial cells. Affected alveoli were partially collapsed and filled with neutrophils, sometimes mixed with mononuclear cells. Collapsed alveoli and infiltrating neutrophils gave the alveolar walls a swollen and increasingly cellualr appearance.

The hilar lymph nodes showed the same histological picture as seen at 12 hours.

Lesions at 2 Days (*Case 961, 962*)

Reddish, rubbery, sunken areas of varying size were scattered in the apical, cardiac and intermediate lobes. The diaphragmatic lobes were free from lesions. Lobar involvement was found in case 962, about three quarters of the apical and intermediate lobes being involved. Emphysematous lobules were found adjacent to the lesions. The cut surface appeared granular. Small amounts of sticky, cloudy mucus were found in the bronchioles, trachea and bifurcation of large bronchi. Markedly swollen pulmonary hilar nodes measured up to 1.5×3 cm in both cases.

Microscopically, the changes were essentially those of an acute bronchopneumonia (Fig. 2 and 3). Lesions had a lobular pattern. Edema was not extensive. Cellular exudates, mostly composed of neutrophils with small amounts of mononuclear cells, were packed in the partially collapsed alveoli. Moderate septal cell hyperplasia was also observed. Marked hyperemia was seen in case 961. Lymphoid hyperplasia, with some plasma cells in which mitotic figures were frequent, was found around the blood vessels. Thickened and hyperplastic peribronchiolar connective tissue elements with infiltrated mononuclear cells and eosinophils were observed (Fig. 4). The bronchiolar mucosa was usually intact, but degenerative and desquamative epithelial changes and infiltrated inflammatory cells in the lamina propria were frequently observed in the small bronchioles and respiratory bronchiolar mucosa. This bronchiolar involvement was prominent in case 962. A moderate amount of exudate, mostly composed of neutrophils and mucus, was found in the lumen of bronchi. Edema in interlobular connective tissue was common. An arterial thrombus was seen in one animal. Solitary lymph follicles showed marked hyperplasia. Diaphragmatic lobes showed frequent atelectasis, but no other significant changes were observed.

Marked lymphoid hyperplasia and neutrophilic foci, in or about the lymph follicles and peritrabecular sinuses, were found in the pulmonary hilar lymph nodes. Endothelial proliferation in the small blood vessels was also observed.

Lesions at 3 Days (*Case 963, 964*)

About two-third of each apical, intermediate, and cardiac, lobes and a part of the anterior portion of the diaphragmatic lobes were involved in both cases (Fig. 5). The lobular lesions were sunken, reddish in color, and rubbery in consistency. The lesions were not necessarily continuous, some non-affected parts being surrounded by lobular lesions, and showing a marble pattern. Small amounts of cloudy and very viscid mucus were observed in the trachea and bifurcation of the large bronchi.

Swollen pulmonary hilar lumph nodes were noted, some being 1.5×3 cm in size.

Microscopically, except some inreased numbers of septal cells seen in the alveoli, essential changes were almost the same as seen in the lesions at 2 days. Sleeve-like peribronchiolar and perivascular lymphoid hyperplasia were marked in the cardiac lobes. Occasional large clumps of fine bluish granules were found in the cytoplasm of septal cells in the alveoli in case 963 (Fig. 6). More abundant alveolar edema was seen in case 964.

Marked lymphoid hyperplasia and many neutrophils infiltrated in or about the lymph follicles were found in the hilar lymph nodes.

Lesions at 4 Days (*Case 776, 777*)

About half of the posterior part of the right diaphragmatic lobe and half of the posterior part of the right apical lobe showed red, elevated, fleshy, consolidated areas in case 776. Red, consolidated, lobular involvument was seen here and there in both diaphragamtic lobes, particulary in the left lung, in case 777. Consolidated portions were either sunken or elevated. Margins of the lesions were very clear and sharp. Small amounts of semi-transparent, very viscid mucus were seen in the large bronchi. Whitish pus filled the bronchioles.

Markedly swollen (about 1.5×3 cm) and juicy pulmonary hilar lymph nodes were observed. Cut edges bulged.

Microscopically, marked perivascular and peribronchiolar lymphoid hyperplasia, with a prominent plasma cell component, was the common feature in both cases (Fig. 7). Partially collapsed alveoli containing about equal proportion of neutrophils, detached septal cells and lymphocytes mixed with coarse, eosinophilic exudate were found everywhere (Fig. 8). Edema was not extensive. Neutrophils were the predominant cellular exudate in the edematous portion. Multinucleated giant cells, with 1 to 15 nuclei in horseshoe-shaped arrangement at the periphery of the cytoplasm, were seen here and there in the alveoli, and sometimes in the peribronchial lymphatics (Fig. 9). In the grossly unaffected lobules next to affected ones, mild perivascular and peribronchiolar monouclear infiltration was observed and the demarcation line between affected and unaffected lobules was clear cut by the interlobular septa. In the epithelial layer of medium-sized bronchioles occasional neutrophilic foci surrounded by an empty space were found (Fig. 10). Slightly taller epithelial cells and slightly increased secretory activity were observed in the bronchioles. Almost pure neutrophils and small amounts of mucus were seen in the bronchioles and bronchi. Slight mononuclear infiltration was seen in the subserosal area, but alveoli beneath the pleura showed comparatively slight changes. In some bronchioles, lymphoid hyperplasia was seen in the lamina propria between the mucosa and the musculature. A thrombus was found in a medium-sized vein in case 776.

Diffuse, marked lymphoid hyperplasia was the predominant picture in the hilar lymph nodes. Markedly swollen and hyperplastic endothelial cells were frequently seen in capillaries and in some arterioles. Occasional multinucleated giant cells were found in the peritrabecular sinuses. Several tiny neutrophilic foci and hemorrhages were found in the cell poor substance and in the peritrabecular sinuses.

Lesions at 8 Days (*Case 799, 800*)

In case 799, a 2.5×4 cm of red, consolidated area at the margin of the left diaphragmatic lobe, and a 2×2 cm consolidated lesion in the middle part of the right diaphragmatic lobe were observed. These were rubbery in consistency. In case 800, consolidated red areas scattered in both diaphragmatic lobes. Large atelectatic areas were seen in both diaphragmatic lobes. Some sunken linear areas were also found. Small amounts of whitish, viscid mucus in the trachea and in the bronchi were observed in both cases.

Markedly swollen and edematous hilar lymph nodes were found.

Microscopically, perivascular and peribronchiolar lymphoid hyperplasia was predominant. Frequent plasma cells and eosinophlls were seen in the hyperplastic lymph tissue. In the affected parts, thickened alveolar walls with hyperplastic septal cells and slight lymphocytic infiltration were found. Most of the alveoli were moderately collapsed and contained numerous detached septal cells with only a few neutrophils. Edema was mild. Some lymph follicles around the bronchioles showd marked hyperplasia and extended into the lamina propria pushing the mucosa inward causing narrowing of the lumen. In the epithelial layer of medium-sized bronchioles occasional neutrophilic foci surrounded by an empty space were found. Multinucleated giant cells were occasionally found in the alveoli. Most of the respiratory bronchiolar epithelial cells were desquamated and the lumens were full of mononuclear cells and coarse, eosinophilic exudates. Occasional organization of exudate was found in the lumen of respiratory bronchioles.

The hilar lymph nodes showed the same histological picture as seen at 4 days.

Lesions at 15 Days (Case 810, 811)

In case 811, there was a 1.0×1.2 cm consolidated gray area in the posterior margin of the left diaphragmatic lobe, and linear, sunken areas were seen at the ventral surface, mostly at the junctures of each lobe of the right lung. Only slight congestion was noted in case 810. Markedly swollen hilar lymph nodes were noted in both cases.

Microscopically, moderate peribronchiolar and occasional perivascular lymphoid hyperplasia were noted. The alveoli were free from exudates. Septal cell hyperplasia and some mononuclear infiltration, chiefly lymphocytes, gave the alveolar walls a swollen appearance. Most of the respiratory bronchiolar epithelial cells were undergoing regeneratin, and appeard taller than normal. In Case 811, organization was obseved occasinally in the small bronchiolar lumen of the posterior margin of the left diaphragmatic lobe.

The changes in the hilar lymph nodes were essentially the same as at 4 days.

Lesions at 21 Days (Case 1095, 1044)

A few linear atelectatic lesions were observed in the anterior part of the right intermediate lobe. No swelling was observed in the hilar lymph nodes.

Microscopically, reticular fibers and collagen were still thick and prominent in the perivascular and peribronchiolar areas. Lymphoid elements were less evident than at 15 days (Fig. 11). Occasional atelectatic foci were observed. Slightly thickened alveolar walls were due to the slight hyperplasia of alveolar epithelial cells and infiltration of a few lymphocytes. No fibrosis of alveolar walls was observed. Occasional organization was seen in the lumen of respiratory bronchioles. Some interlobular septa showed increased blood vessels and torturous dense collagen. Increased, dense adventitial tissue was seen in some small arteries beneath the visceral pleura.

Lymphoid hyperplasia and marked germinal centers were noted in the hilar lymph nodes.

Lesions at 4 Days after Challenge (Case 1314)

Red, fleshy, lobular, consolidated, sunken areas with some adjacent emphysema were found in about two-third of the apical lobes, and a few of such foci were seen in the intermediate lobe and anterior parts of both diaphragmatic lobes. The right lung was more affected. Grayish-brown, raised foci scattered here and there throughout the lungs.

Markedly swollen hilar lymph nodes were found.

Microscopically, marked sleeve-like lymphoid hyperplasia around the bronchioles and the vascular walls was observed. Marked peribronchial and perivascular reticular fiber proliferation was apparent (Fig. 12). Partially collapsed alveoli contained many detached and hyperplastic septal cells and lymphocytes. Neutophils were found mostly in bronchioles with only a few in alveoli. The bronchiolar mucosa was generally intact, in some parts tiny neutrophilic foci surrounded by vacant clear space in the mucosal epithelial layer was observed. The bronchi and large bronchioles contained moderate amounts of mucus and degenerating neutrophils. The changes were comparable to those observed at 8 days after inoculation.

Marked lymphoid hyperplasia was noted in the hilar lymph nodes.

Lesions at 9 Days after Challenge (Case 1349)

A few tiny, linear atelectic foci were found here and there. Moderately swollen hilar lymph nodes were observed.

Microscopically, the changes were comparable to that of 21 days after inoculation.

Non-pulmonary Organs (Spleen, Liver, Kidney, Stomach, Intestine, Urinary Bladder, Brain, and Regional Lymph Nodes)

Other organs were free from lesions in the whole series.

Control

No abnormalities were found in the lungs of 3 normal control pigs and the other pig killed 2 days after receiving normal yolk sac suspension.

Summary of the Histopathology of the Disease

Characteristic features of this pneumonia were initiated by simultaneous neutrophilic infiltration and increased peribronchiolar and perivascular connective tissue. These changes intensified along with neutrophilic infiltration, desquamation of alveolar and respiratory bronchiolar epithelial cells, septal cell hyperplasia, and slight edema in the alveoli. The resulting exudates blocked the air passages at respiratory and terminal bronchioles inducing atelectasis. Bronchial and bronchiolar involvement was usually minimal. Neutrophilic infiltration reached its peak at 3 days after inoculation, while perivascular and peribronchiolar lymphoid hyperplasia progressively developed and reached its peak at 4 to 8 days after inoculation. Meanwhile, Neutrophilic infiltration decreased from the 4th day onward, and disappeared entirely by 15 to 21 days after inoculation. Lymphoid hyperplasia also decreased progressively after 8 days, and a mild degree of perivascular and peribronchiolar lymphoid hyperplasia and mild septal cell hyperplasia remained at 15 to 21 days after inoculation. Regeneration of the damaged respiratory bronchiolar epithelium usually took place within this period, however, organization was occasionally seen in the respiratory bronchioles which may be explained by the occurence of prolonged atelectasis. Otherwise the lung tissues recovered virtually without evidence of earlier damage.

Virus Staining

Smears and sections were prepared from lungs and pulmonary lymph nodes in each case and stained with Macchiavello, Pollak, Feulgen, Feulgen NAH and PAS stains. Materials from both infected and normal yolk sac membranes of 5-day-old embryonated eggs were examined for comparison.

With Machiavello stain, intracytoplasmic elementary bodies were identified as red, fine and spherical bodies in neutophils and in a few mononuclear cells in both sections and smears from lungs and pulmonary hilar lymph nods of infected animals. The elementary bodies appeared within 4 days after inoculation, particulary prominent in the lungs 2 to 3 days after inoculation. Occasionally, macrophages were seen containing phagocytized neutrophils within which many elementary bodies were observed. However, these bodies were never found in the bronchial epithelial cells. Elementary bodies were also identifiable by Feulgen and Feulgen NAH stain. PAS stain is considered unsatisfactory, because non-virus substances occasionally gave staining characteristics hardly differentiable positively from the virus particles.

It is of interest that, with Pollak stain, uniformlly spherical bodies were constantly detectable within the cytoplasm of neutrophls in both section and smear preparations from infected lungs and pulmonary lymph nodes. They were also dectected in smear preparations of infected yolk sac membranes; however, it is difficult to differentiate these bodies from the contaminated yolk material which took a bright red color in histological preparations. Therefore, this staining method is not suitable for histological specimens prepared from yolk sac membranes. Occasionally in lung smear preparations, distemper-type cytoplasmic bodies, inclusion-like, were observed in the cytoplasm of neutrophils and macrophages by Pollak stain. The granules of eosinophils also take red color by this staining method, but there is no difficulties in differentiation; their size are much larger than elementary bodies, and they were refractile and packed togeter.

The elementary bodies were positively identified from 1 to 4 days after inoculation by Macchiavello's stain, but not at 8 days. In case 1314, which was sacrifieed at 4 days after challenge in Experiment II, elementary bodies were not detectable by Macchiavello's staining technique.

Microbiology

Virus Recovery from Lung

Attemps for virus isolation were made on lung specimens of animals killed at 4, 8 and 15 days respectively after inoculation. 10% lung suspensions to which was added Tyrothricin, Sulfadiazine, and Streptomycin (TSS solution (4)) were kept in the refrigerator (4 C) overnight. 0.5 ml doses were inoculated into the yolk sacs of 5 to 7-day-old embryonating eggs, 6 eggs being used for each sample. Virus was recovered in the 4 and 8 day infection groups, but not in the 15-day group. The results are shown in Table II.

TABLE II

Virus recovery from the Lungs of experimentally infected pigs

Days after		Pig No.	Result
Infection	Rechallenge		
4		776 777	+ +
8		799 800	+ +
15		810 811	– –
	4	1314	+
	9	1349	–

10% lung suspensions which contained TSS solution were prepared from cases 1314 and 1349, 4 and 9 days after challenge, respectively. 0.5 ml doses were inoculated into the yolk sac of 5-day-old embronating eggs, 6 eggs being used for each sample. Only one of the 6 which received lung suspension from case 1314 died on 6th day after inocolation with positive results. None of the remaining embryos died within the 14-day observation period. The results are shown in Table II.

Attempt of Virus Isolation from Heart Blood

In Experiment II, two pigs were selected at random and each was bled 10 ml of blood from the right heart at 1, 2, 3 and 4 days after inoculation, respectively. Virus isolation was attempted by inoculating defibrinated blood into 5 to 7-day-old embryonating eggs, 0.5 ml of defibrinated blood being inoculated into each yolk sac. No less than 12 eggs were used for each sample. At least two blind passages were made. Results are invariably negative.

Routine Microbioloy for Lung Specimens

Thioglycollate medium, blood agar plate, PPLO medium enriched with 10% normal horse serum and 1% yeast hydrolysate were used for routine bacteriology. A few colonies of hemolytic streptococci were found on the culture of a lung specimen from case 964. No bacterial growth was observed in the other cases (Table I).

DISCUSSION

The pathogenicity of ovine pneumonia virus for pigs inoculated by the intratracheal route was definite and consistent. It produced the same pathological picture as seen in the experimentally infected sheep (3, 9), but it had some similarity to VPP, too. Peribronchiolar and perivascular lymphoid hyperplasia was apparent at the 2nd day, reaching its maximum at 4 to 8 days after inoculation. It subsided afterwards but was still demonstrable 21 days after inoculation. From the 8th day onward, the histological picture was similar to VPP (5, 6, 7). However, the pneumonia produced in this study was typical bronchopneumonia, with peribronchial and perivascular lymphoid hyperplasia at the early stage of infection, while experimentally produced VPP (5, 7) would not develop alveolar or bronchiolar involvement within 10 days after inoculation. Therefore, it is not difficult to postulate the difference in two infections. Moreover, the microbiological studies on experimental pigs showed that artificially produced pneumonic lungs were free from becterial contamination, except in case 964, a few colonies of hemolytic streptococci were found on the culture of lung speciemens. Three normal and one yolk control pigs were free from pneumonia. From these facts it is obvious that the experimentally produced pneumonia in this study was caused by ovine pneumonia virus.

In the present study typical pneumonia and respiratory symptoms were consistently produced by sheep pneumonia virus in swine. Since the infection dose used was conceivably larger than that of natural infection, and the inoculum was prepared from infected yolk sac membrane, the question may arise as to whether the pneumonia produced was due to some preformed toxic substance, *e.g.*, virus toxin, rather than a genuine sequence of virus multiplication. It will be noted that some animals showed fever response and respiratory signs 2 days after inoculation, and elementary bodies were generally most abundant in lung smears 2 to 3 days after inoculation. If the pneumonia was caused by the preformed toxin in yolk sacs, then the fever response and respiratory signs should appear earlier. Again, the appearance of elementary bodies in this study agrees well with that of experimental sheep penumonia (3, 9) so far as time factor is concerned. Serological evidence was incomplete to permit definite conclusion to drawn from this study, but the available data favors the view point that the clincal picture observed is the result of virus multipication in lungs as well as to virus toxin activity.

Omori *et al.* (8) produced pneumonia in a single swine with goat pneumonia virus. They concluded that since the virus could produce severe pneumonia and respiratory symptoms with elevated complement fixing antibody titer, the pig is susceptible to this virus infection. Unfortunately, no detailed histopathological findings were described.

Perivascular lymphoid hyperplasia appeared earlier than peribronchiolar lymphoid hyperplasia, and was a constant feature in the whole series. Our result confirms the findings of Boidin *et al.*(3), and Dungworth et al. (9), who observed similar changes in lambs given this sheep pneumonia virus. It differs from the findings of Ishitani *et al.* (10, 11) who reported that in natural and experimental goat virus pneumonia, the peribronchiolar, instead of perivascular, lymphoid hyperplasia was the major pathological feature. It is not clear whether the difference is due to the different nature of the virus itself or to the host.

Ishitani *et al.* (10) also reported an increase of reticular fibers within the alveolar walls and the peribronchiolar spaces in their cases of goat pneumonia. In the present study, however, reticular fiber proliferation was only observed in the peribronchiolar and perivascular areas, but was never found in the alveolar walls.

Of interest was the dependence of lung lesions on inoculation posture. When animals were inoculated in their natural standing position, the lesions were distributed in the apical, intermediate and cardic lobes; but if held supine during inoculation, the lesions were found mainly in the diaphragmatic lobes. This finding coincides with the reports of Ishitani *et al.* (11). Droplet infection might have resulted in uniform distribution of the lung lesions, had atomizing facilities made available to us for experimentation.

The two pigs (1314, 1349) which were killed at 4 and 9 days after challenge showed lesions comparable to those killed at 8 and 21 days after primary inoculation in Experiment I and II. This may be interpreted as due to an increased tissue immunity.

Histologically no significant lesions were detectable in other viscera. Ishitani *et al.* (11) reported that the changes of the viscera in experimental cases were slighter than those of field cases. The changes in the lungs of field cases showed more diffused lesions. Based on these facts they suggested possible hematogenic distribution of viruses in field cases. In the present study, virus isolation from circulating blood in embryonated eggs within 4 days after inoculation was unsuccessful.

SUMMARY

1) Sheep pneumonia virus (Boidin's strain SPB) was definitely pathogenic for pigs by the intratracheal route. It produced characteristic pneumonia and clinical symptoms identical to those seen in the sheep host.

2) Acute bronchopneumonia appeared in the early stage followed by progressive lymphoid hyperplasia around the bronchioles and vascular walls. A histological resemblance to virus pneumonia of pigs occurred from the 8th day onward after inoculation. Lung lesions were primarily lobular in nature, by confluence occasional lobar involvement was observed.

3) Neutrophils vere the earliest inflammatory cells in response to the virus incoulation. Intracytoplasmic elementary bodies were usually found in neutrophils and mononuclears in the early stage of infection.

4) Virus was recovered from lungs 4-8 days after primary inocculation, but not 15 days. In challenge experiments, virus was recovered from lungs at 4th day, but not at 9th day, after inoculation. Viremia was not demonstrable within 4 days after primary inoculation.

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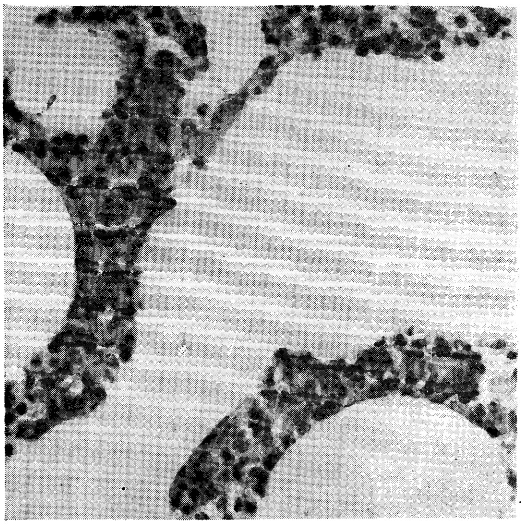

Fig. 1. Acute inflammatory cell infiltrations in the swollen alveolar walls. Alveoli are still free from exudates. 12 hours after inoculation. H & E stain, ×240.

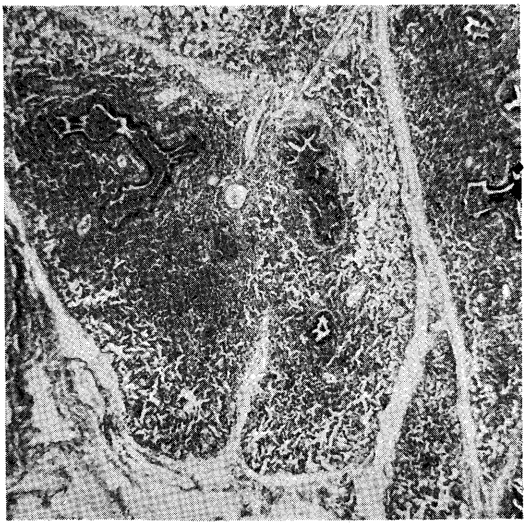

Fig. 2. Acute bronchopneumonia in the early stage of the infection. Note almost pure neutrophilic exudate in the terminal bronchioles and alveoli. 2 days after inoculation. H & E stain, ×30.

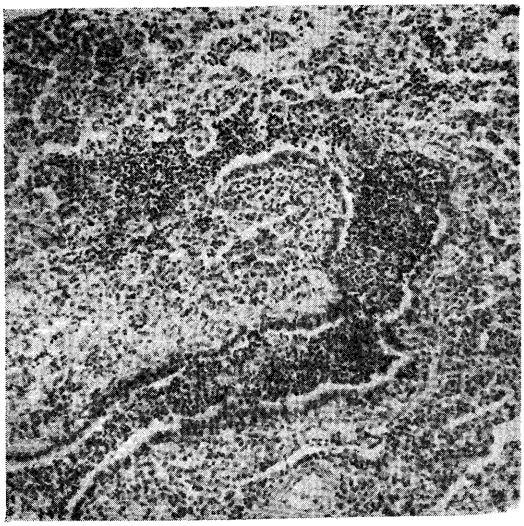

Fig. 3. Almost pure neutrophilic exudate blocking the respiratory bronchiole and alveolar duct. Many adjacent alveoli collapsed. 2 days after inoculation. H & E stain, ×110.

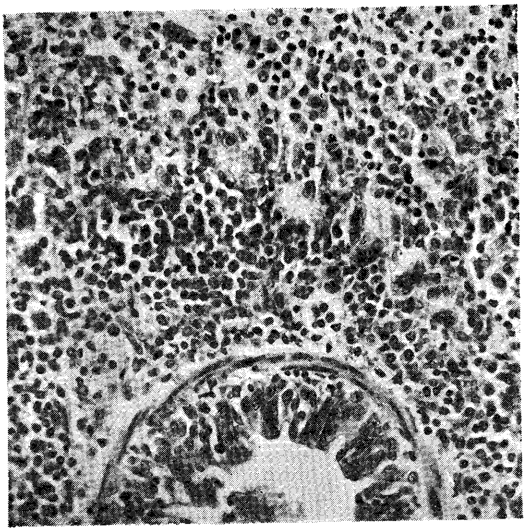

Fig. 4. Early lymphocytic infiltration in the peribronchiolar tissue. Septal cells and neutrophils are seen in the alveoli. 2 days after inoculation. H & E stain, ×240.

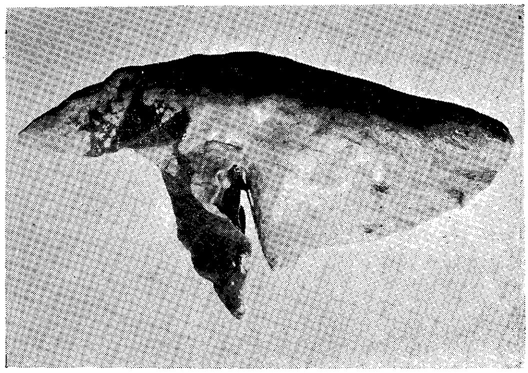

Fig. 5. Pattern of involvement in the apical and intermediate lobes. The diaphragmatic lobe was free from gross lesions. The animal was inoculated in the standing position. 3 days after inoculation.

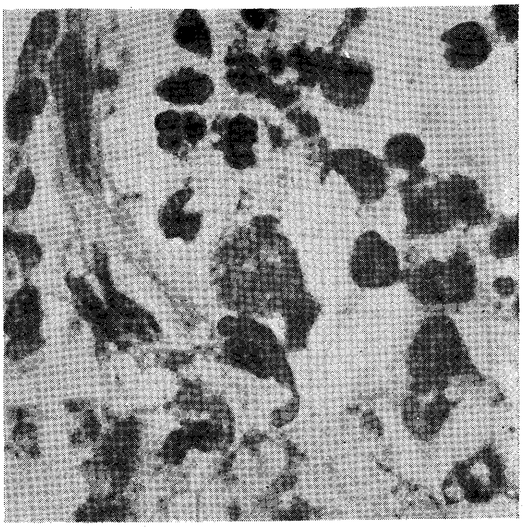

Fig. 6. A basophilic mass, presumably a virus-filled cell, seen in the center of the field. 3 days after inoculation. H & E stain, ×1,000.

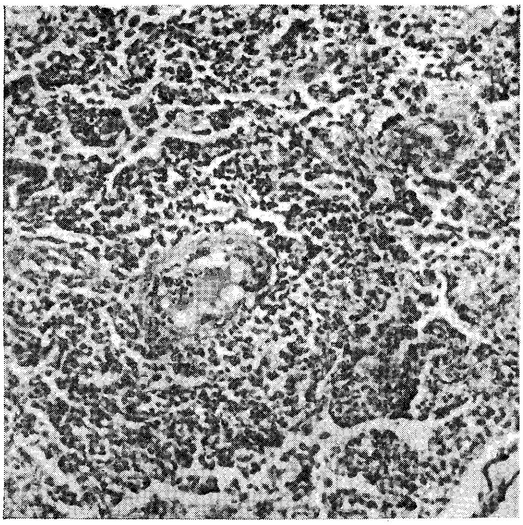

Fig. 7. Sleeve-like lymphoid hyperplasia seen in the perivascular area. Detached septal cells and neutrophils can be seen in the alveoli. 4 days after inoculation. H & E stain, ×200.

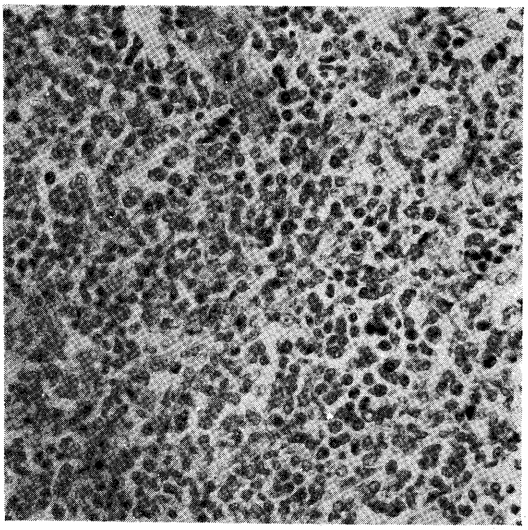

Fig. 8. Infiltrated neutrophils, detached cells and lymphocytes in the alveoli. 4 days after inoculation. H & E stain, ×240.

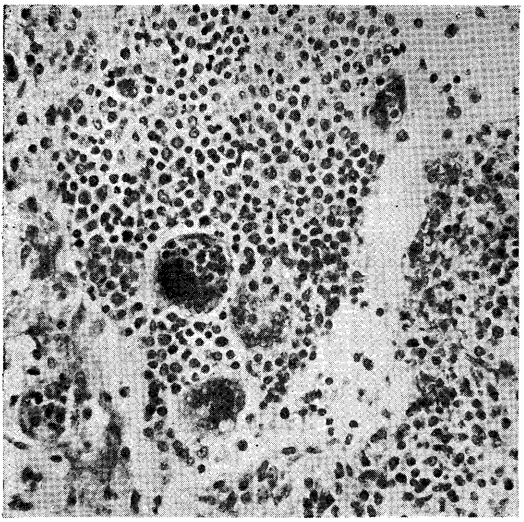

Fig. 9. Multi-nucleated giant cells, which are engulfing lymphocytes, are seen in the peribronchial lymphatics. 4 days after inoculation. H & E stain, ×200.

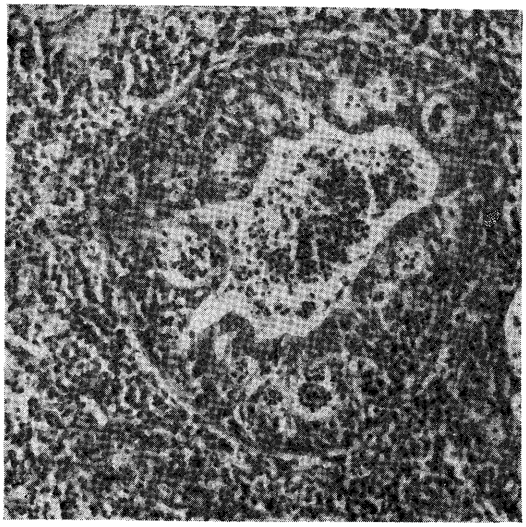

Fig. 10. Neutrophilic aggregated foci in the mucosal epithelium of a bronchiole. Peribronchiolar lymphoid hyperplasia and neutrophilic exudates were seen in the alveoli and the bronchiolar lumen. 4 days after inoculation. H & E stain, ×100.

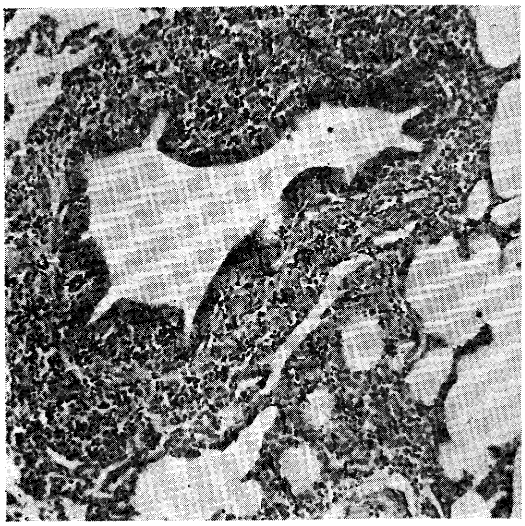

Fig. 11. Peribronchiolar lymphoid hyperplasia still demonstrable at 21 days after inoculation. H & E stain, ×60.

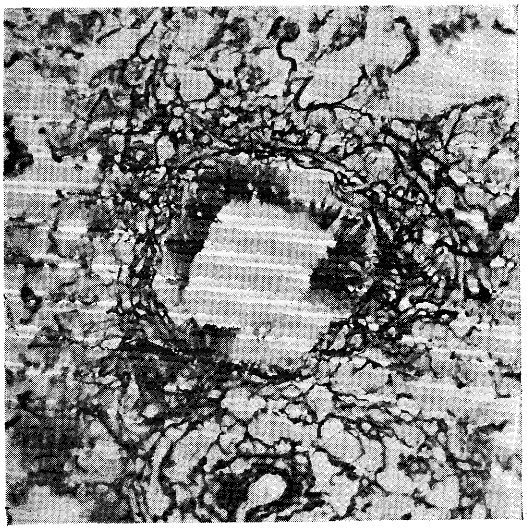

Fig. 12. Peribronchiolar and perivascular reticular fiber proliferation. 4 days after challenge. Reticular fiber stain after Bielshowsky-Foot method, ×120.